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Polyphenol content and antioxidant capacity of the skin extracts of berries from seven biotypes of the Greek grapevine cultivar Korinthiaki Staphis (*Vitis vinifera* L.)

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Abstract

The polyclonality of a grapevine cultivar plays a significant role in the quality of the viticultural products it yields, especially when age-old grapevine cultivars such as Korinthiaki Staphis are entailed. The aim of the present study was to determine the polyphenol content and antioxidative capacity of the berry skins of seven (7) biotypes -possibly clones of the grapevine cultivar Korinthiaki Staphis (*Vitis vinifera* L.). For the purposes of the present study, it is worth noting at this point that all seven biotypes had been cultivated in the same geographic location and under the same climate and soil conditions. In view of the study's aim, the biotypes were studied using high performance liquid chromatography (HPLC) coupled with a diode array detector and spectrophotometer. The results revealed that the levels of both polyphenol content and antioxidant capacity were high in all biotypes. Statistically significant differences between and among the biotypes were duly recorded: (a) Biotype KS15 exhibited a high concentration in total anthocyanins, total flavanols, total flavonoids, acidity, and total soluble solids; (b) biotype KS6 exhibited a high concentration in total soluble solids, total flavanols, epicatechin, procyanidins B1 and B2, trans-resveratrol, and piceid; and (c) biotype KS1 exhibited a high concentration in quercetin, rutin, catechin, epicatechin, trans-resveratrol, and piceid; and the highest concentration in the phenolic aldehyde vanillin. Both polyphenolic content and antioxidant capacity are biotype-dependent. Thus, when striving for products of exceptional quality it is crucial for viticulturists to exploit the appropriate biotypes of Korinthiaki Staphis. Research and results on the studied biotypes suggest that KS15, KS1, and KS6, individually or in combination, are the most suitable ones for the establishment of productive vineyards.

Keywords: antioxidant capacity, biotypes, clonal selection, grape skins, polyphenols, *Vitis vinifera* L.
Abbreviations: AFLP_Amplified Fragment Length Polymorphism; DPPH_2,2-diphenyl-1-picrylhydrazyl; HPLC_High-Performance Liquid Chromatography; FRAP_Ferric Reducing Antioxidant Power; PDO_Protected Designation of Origin

Introduction

In recent years, nutrition experts have been touting the consumption of fruits and vegetables as a vital component of a wholesome nutrition regime leading to physical fitness and the prevention of a number of diseases. In view of the nutritionists' recommendations, the market stimulated among consumers a keen interest in alcohol-free grape products such as grapes and raisins. The overwhelming majority of the biological benefits human health derives from such products is closely associated to those products' antioxidant capacity stemming from the presence of polyphenolic compounds (Di Lorenzo et al., 2016).

Recently, attention has been focused on polyphenolic compounds and their antioxidant properties. Both, found on the berry skin of mainly red grapevine cultivars, are transferred to the wines as well as the raisins produced (Negro et al., 2003; Kallithraka et al., 2006; Arnous and Meyer, 2008; Iacopini et al., 2008; Poudel et al., 2008; Breksa et al., 2010; Katalinic et al., 2010; Pantelić et al., 2016). One such case is Korinthiaki Staphis, a grapevine cultivar indigenous to Greece.

Grapevine cultivar Korinthiaki Staphis (*Vitis vinifera* L.) is considered one the oldest cultivars of the Greek vineyard, so old, in fact, that its cultivation may date back to Greek antiquity. In Greek viticulture and from an ampelographic, viticultural, social, and economic point of view, Korinthiaki Staphis ranks among the top grapevine cultivars: for centuries on end, the black currant (Korinthiaki Staphis) was the dominant export of Greece, sustaining the economy and creating jobs. The first written records on the black currant trade can be traced back to the early 14th century, with the most accurate one being given by Pegolotti (1340) in his book *Pratica della mercatura* [The Merchant's Handbook]. It is certain that the Greeks of classical antiquity knew of the dietary and nutritional merits of raisins and currants, possessed the know-how for grape drying, and engaged in the production and trade of those products. Still, it is far from certain that the grapevine cultivar described by Aristotle as having "small berries lacking nuclei"; and Hippocrates's 'stafiditis oenos' were related to the actual Korinthiaki Staphis grapevine cultivar.

Today, Greece and, more specifically, the regions of western Peloponnese and the Ionian Islands, constitute the main

Korinthiaki Staphis hub worldwide, with cultivation encompassing a total surface area of approximately 15,000 ha and a production that hovers at 20,000 tons of black currants (2016).

Due to its ancient lineage and cultivation, Korinthiaki Staphis goes by numerous synonyms (Guillon, 1895; Molon, 1906; Viala and Vermorel, 1909; Krimbas, 1943; Davidis, 1982; Vlachos, 1986; Stavrakakis, 2010; Robinson et al., 2012). Its polyclonal synthesis has been confirmed by a study relying on the ampelographic description and the AFLP molecular method through the identification/discrimination of twenty (20) biotypes/possible clones (Stavrakaki and Biniari, 2016).

Most studies investigating the polyphenolic content of grapevine varieties turn to plant material -grapes, wines, raisins- usually originating in productive vineyards where several clones of usually polyclonal varieties are cultivated together. Nevertheless, Greece is a country where varieties present great genetic diversity and clones of Greek grapevine varieties have yet to be certified. Thus, clonal selection raises a major issue when it comes to the production of quality wines and raisins: the clones of a single variety may differ so much in productive capacity and properties as to yield products whose organoleptic properties are discrete (Stavrakakis, 2013).

The aim of the present study was to determine and evaluate, by means of spectrophotometry and HPLC, the berry skin content in polyphenols and antioxidant properties of seven biotypes of Korinthiaki Staphis (*Vitis vinifera* L.).The study is part of a broader project researching the polyclonal synthesis of the Korinthiaki Staphis grapevine cultivar with a view to recommending the clones best suited for production and exploitation of high-quality currants (black raisins) as well as wines.

Results and Discussion

The results (mean value and standard error) of each compound obtained from the skins of the studied biotypes of Korinthiaki Staphis are shown in Tables 2-10. The statistically significant differences found between the studied biotypes and for each compound analyzed have been highlighted and flagged with discrete super indexes.

Grape and berry mechanical properties and characters of the must

The average length and weight of the grapes of the Korinthiaki Staphis biotypes studied respectively ranged between 12.5-14.33 cm and 56.33-129.66 g. No statistically significant difference between the biotypes ensued. Biotype KS17 exhibited the highest average berry length and width as well as the highest average weight per 50 berries without any statistically significant difference from biotypes KS1 and KS20 (Table 2). As to the characters of the must and in terms of statistically significant differences, biotype KS8 exhibited the highest pH when compared to all other biotypes. Biotypes KS8 and KS6 both registered the highest concentration of total soluble solids and, consequently, a statistically significant difference from the remaining biotypes studied. With regard to total titratable acidity, biotype KS2 exhibited the highest concentration and KS6 the lowest (Table 2).

Polyphenolic compounds in berry skin
Flavonoids

Flavonols: Between qualitatively and quantitatively detected luteolin, quercetin, and rutin, it was rutin which presented a significantly higher concentration in all biotypes of Korinthiaki Staphis, ranging from 147.750 to 561.882 μg/g skin. The finding confirmed previous studies (Fabani et al., 2017) which had found that rutin is the main flavonoid in berry skin (Table 3). Among the biotypes of Korinthiaki Staphis studied in the current experiment, the highest concentration of rutin was observed in biotype KS1 with a statistically significant difference from the rutin concentration observed in all other biotypes. Still, the rutin and quercetin concentration in all Korinthiaki Staphis biotypes studied was higher than that of certain wine grape varieties (Iacopini et al., 2008) and some raisin grape ones (Fabani et al., 2017). Regarding luteolin concentration in berry skin, biotype KS20 registered the highest score with a statistically significant difference from the remaining biotypes studied. Biotypes KS1, KS15, and KS20 presented the highest quercetin concentrations, with a statistically significant difference from the remaining biotypes (Table 3).The fact that three of the biotypes, i.e., KS1, KS15, and KS20, registered a high concentration in the flavonols luteolin and quercetin is interesting in itself but becomes an all-important finding when linked to a recent study which has revealed that a high concentration of luteolin and quercetin in raisins may have significantly beneficial effects on human health (Carughi, 2009).

Flavanols: The berry skin of biotype KS1 exhibited the highest concentration in catechin. And although the concentration of epicatechin in the biotypes studied was significantly lower than the concentration in catechin, it was quantified as being at satisfactory levels in comparison to other varieties, such as Arizul, Superior, Flame, and Sultanina (Fabani et al., 2017). Biotype KS6 exhibited the highest concentrations in epicatechin and in the procyanidinsB1 and B2. Further, KS6's epicatechin concentration showed no statistically significant difference from that of biotype KS17. Biotype KS15 exhibited the higher concentration in total flavanols but no statistically significant difference from biotypes KS6 and KS2 (Table 4). The high concentration of those three flavanols in the berry skin contributes to the quality of the produced raisins: catechins and procyanidins may indeed decrease when affected by oxidative reactions (Franke et al., 2004; Harnly et al., 2006). Nevertheless, they do remain at satisfactory levels after post-harvest dehydration (Moreno et al., 2008).

Anthocyanins: Five anthocyanins and, more specifically, delphinidin, cyanidin, petunidin, peonidin, and malvidin, were identified and quantified in all the Korinthiaki Staphis biotypes studied. Biotype KS2 exhibited the highest concentrations in the individual anthocyanins delphinidin, petunidin, peonidin, and malvidin; and in total anthocyanins. It showed no statistically significant difference from biotype KS15. The highest concentration of cyanidin was recorded in biotype KS17 but it registered no statistically significant difference when compared to that of biotypes KS2, KS8, and KS15 (Table 5). Biotypes KS2 and KS15 both presented high

Table 1. Biotypes of Korinthiaki Staphis (*Vitis vinifera* L.) studied and cultivation center.

a/a	Code	Biotype [a]	Berry skin color [b]	Cultivation center
1	KS 1	Korinthias ntopio	N	Corinth
2	KS 2	Kefallinias	N	Cefallonia
3	KS 6	Zakinthou prwimo	N	Zante
4	KS 8	Zakinthou ntopio	N	Zante
5	KS 15	Vostitsa	N	Aigialeia (Achaia)
6	KS 17	Vostitsa	N	Korakochori (Elia)
7	KS 20	Pyrgou ntopio	N	Elia

a: Transliteration of the variety's name from Greek into Latin (ELOT 743/ISO 843:1997). b. N: noir (black)

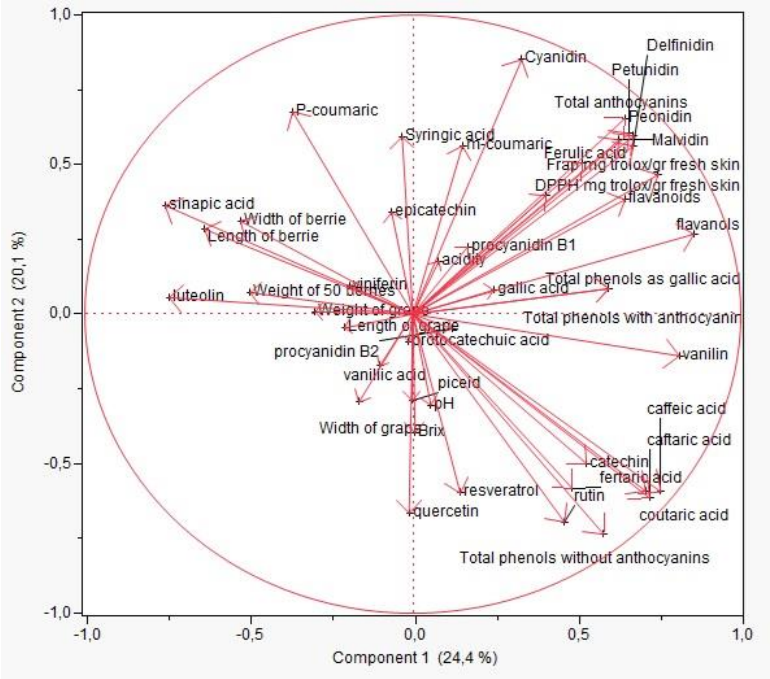

Fig 1. Evaluation of the 45 variables/measurements studied and their contribution to the variability of the biotypes studied.

Table 2. Characters of the must, grape, and berry mechanical properties.

Biotypes	Total Soluble solids (Brix)	pH	Total Titratable Acidity (g/L tartaric acid)	Average Length of Grape (cm)	Average Width of Grape (cm)	Average Weight of Grape (g)	Average Length of Berry (cm)	Average Width of Berry (cm)	Average Weight of 50 Berries (g)
KS 1	24.2±0.11 b	3.79±0.008 b	5.26±0.43 bc	14.33±0.16 a	8±0.57 abc	112.33±14.11 a	8.38±0.10 b	8.41±0.25 b	31±1.73 a
KS 2	23.7±0.05 b	3.73±0.006 bc	7.05±0.22 a	12.66±0.88 a	8.16±0.72 ab	88.66±15.16 a	7.13±0.08 c	7.17±0.17 c	19±2.64 b
KS 6	27.46±0.29 a	3.62±0.06 c	3.73±0.01 d	13±0.50 a	6.33±0.44 bc	101.33±16.75 a	7.9±0.22 bc	7.77±0.26 bc	18±0.57 b
KS 8	28±0.11 a	4.08±0.006 a	4.46±0.03 cd	12.5±0.76 a	5.166±0.16 c	56.33±6.36 a	8.01±0.42 bc	8.04±0.38 bc	15.66±0.88 b
KS 15	23.66±0.33 b	3.72±0.006 bc	4.83±0.22 bcd	13.16±0.60 a	7.5±0.5 abc	79.33±18.47 a	7.75±0.10 bc	7.69±0.10 bc	21±0.57 b
KS 17	21.9±0.05 c	3.62±0.006 c	5.66±0.22 b	14.33±0.88 a	6.5±1.04 abc	129.66±17.74 a	9.91±0.26 a	9.84±0.33 a	36±0.57 a
KS 20	24±0.28 b	3.72±0.04 bc	5.83±0.16b	13.83±0.16 a	9.33±0.33 a	110.66±17.14 a	8.93±0.33 ab	8.52±0.15 b	33±1.73 a

Mean values (Mean ± SE) in the same column but assigned different letters (a–d) are significantly different according to Tukey's range test at P≤0.05.

Table 3. Individual flavonols in berry skin.

Biotypes	Luteolin (μg/g skin)	Quercetin (μg/g skin)	Rutin (μg/g skin)
KS 1	1.625±0.13 c	3.866±0.13 a	561.882±1.19 a
KS 2	0.965±0.22 c	2.610±0.22 bc	276.954±2.20 b
KS 6	1.484±0.07 c	1.581±0.07 cd	215.872±12.92 c
KS 8	1.556±0.12 c	1.400±0.12 cd	281.875±3.59 b
KS 15	3.790±0.17 b	3.096±0.17 ab	198.443±8.34 cd
KS 17	3.177±0.05 b	0.566±0.05 d	173.061±12.24 de
KS 20	6.893±0.58 a	3.963±0.58 a	147.750±5.16 e

Mean values (Mean ± SE) in the same column but assigned different letters (a–e) are significantly different according to Tukey's range test at P≤0.05.

Table 4. Total and individual flavanols in berry skin.

Biotypes	Total flavanols (µg/g skin)	Individual flavanols			
		Catechin (µg/g skin)	Epicatechin (µg/g skin)	Procyanidin B1 (µg/g skin)	Procyanidin B2 (µg/g skin)
KS 1	1534±47.34 b	50.01±0.76 a	0.541±0.04 abc	1.748±0.11 b	0.685±0.08 a
KS 2	1675±73.32 ab	43.89±0.03 b	0.477±0.02 bc	1.821±0.14 b	0.142±0.007 b
KS 6	1660±68.12 ab	21.273±1.46 c	0.631±0.01 ab	2.917±0.20 a	0.794±0.11 a
KS 8	1554±32.33 b	11.720±0.30 e	0.487±0.03 bc	2.228±0.22 ab	0.713±0.08 a
KS 15	1807±56.00 a	18.41±0.66 cd	0.254±0.11 c	2.431±0.13 ab	0.604±0.02 a
KS 17	1525±19.05 b	16.27±1.79 de	0.831±0.13 a	2.081±0.24 ab	0.624±0.02 a
KS 20	1039±39.83 c	18.909±0.003 cd	0.350±0.01 bc	1.748±0.20 b	0.276±0.005 b

Mean values (Mean ± SE) in the same column but assigned different letters (a–e) are significantly different according to Tukey's range test at P≤0.05.

Table 5. Total and individual anthocyanins in berry skin.

Biotypes	Total anthocyanins (µg/g skin)	Individual anthocyanins				
		Delphinidin (µg/g skin)	Cyanidin (µg/g skin)	Petunidin (µg/g skin)	Peonidin (µg/g skin)	Malvidin (µg/g skin)
KS 1	578.75±50.35 c	8.19±1.18 cd	30.29±4.16 c	12.05±1.47 d	85.09±7.9 bc	108.87±8.32 c
KS 2	1131.76±26.24 a	37.78±3.99 a	86.51±7.9 a	47.09±4.5 a	172.54±12.59 a	318.18±22.86 a
KS 6	632.97±37.36 c	18.48±0.80 bc	70.22±3.9 b	21.76±0.99 dc	95.07±4.32 bc	119.75±4.85 c
KS 8	525.51±31.92 c	13.1±2.68 cd	83.63±16.2 ab	15.95±2.77 cd	93.17±16.32 bc	61.23±10.43 c
KS 15	1011.91±50.91 ab	31.06±4.70 ab	99.91±4.16 a	39.73±5.63 ab	169.45±17.2 a	253.32±28.22 ab
KS 17	939.88±36.32 b	20.14±2.13 bc	100.71±92 a	28.79±2.18 bc	139.59±9.8 ab	217.22±8.73 b
KS 20	335.32±8.45 d	5.05±0.65 d	39.57±4.455 c	7.46±0.83 d	63.59±5.44 c	59.13±5.29 c

Mean values (Mean ± SE) in the same column but assigned different letters (a–d) are significantly different according to Tukey's range test at P≤0.05.

Table 6. Hydroxybenzoic acids in berry skin.

Biotypes	Gallic acid (µg/g skin)	Protocatechuic acid (µg/g skin)	Syringic acid (µg/g skin)	Vanillic acid (µg/g skin)
KS 1	0.385±0.04 b	0.226±0.01 b	5.822±0.31 b	7.197±0.25 a
KS 2	0.319±0.01bc	0.135±0.003 d	3.350±0.03 b	1.747±0.11 d
KS 6	0.927±0.05 a	0.363±0.01 a	7.158±0.01 ab	5.962±0.19 b
KS 8	0.475±0.04 b	0.203±0.003 bc	5.840±0.28 b	7.215±0.08 a
KS 15	0.461±0.01 b	0.175±0.01 cd	5.840±0.41 b	6.703±0.44 ab
KS 17	0.442±0.04 b	0.218±0.01 bc	11.882±2.73 a	6.209±0.20 ab
KS 20	0.186±0.009 c	0.155±0.005 d	3.222±1.14 b	4.801±0.09 c

Mean values (Mean ± SE) in the same column but assigned different letters (a–d) are significantly different according to Tukey's range test at P≤0.05.

Table 7. Hydroxycinnamic acids in berry skin.

Biotypes	Sinapic acid (µg/g skin)	Caffeic acid (µg/g skin)	P-coumaric acid (µg/g skin)	m-coumaric acid (µg/g skin)	Ferulic acid (µg/g skin)	Caftaric acid (µg/g skin)	Coutaric acid (µg/g skin)	Fertaric acid (µg/g skin)
KS 1	3.69±0.09 d	147.40±5.30 a	1.05±0.03 b	1.04±0.14 b	2.48±0.24 bcd	467.28±21.16 a	9.99±0.53 a	19.39±0.08 a
KS 2	3.81±0.03 d	114.27±1.27 b	1.11±0.01 b	0.69±0.106 c	3.62±0.55 b	326.89±6.20 bc	7.65±0.26 b	11.19±0.21 b
KS 6	5.26±0.38 d	95.003±5.11 c	1.51±0.09ab	0.44±0.001 cd	2.34±0.15 cd	281.06±22.41 cd	6.42±0.34 b	6.93±0.37 d
KS 8	3.91±0.54 d	63.585±0.13 d	1.50±0.20ab	0.36±0.01 cd	1.80±0.06 d	230.44±5.02 de	4.71±0.30 c	7.91±0.34 cd
KS 15	7.38±0.42 c	102.14±2.91bc	1.52±0.04ab	1.09±0.006 b	5.43±0.24 a	344.41±10.08 b	7.25±0.01 b	8.91±0.03 c
KS 17	9.90±0.14 b	36.97±1.54 e	2.42±0.59a	1.94±0.04 a	3.57±0.14 bc	146.62±0.73 f	3.81±0.02 c	7.89±0.20 cd
KS 20	11.67±0.53a	50.60±0.9 de	1.56±0.01ab	0.33±0.04 d	2.01±0.04 d	188.87±7.27 ef	4.52±0.18 c	6.97±0.25 d

Mean values (Mean ± SE) in the same column but assigned different letters (a–f) are significantly different according to Tukey's range test at P≤0.05.

Table 8. Stilbenes and phenolic aldehyde in berry skin (Mean ± SE).

Biotypes	Stilbenes			Phenolic aldehyde
	Trans-resveratrol (µg/g skin)	ε – viniferin (µg/g skin)	Piceid (µg/g skin)	Vanillin (µg/g skin)
KS 1	56.340±4.15 a	8.464±0.72 c	15.325±0.94 ab	1.189±0.07 a
KS 2	31.723±0.76 b	12.046±0.75 ab	10.432±0.57 c	0.972±0.02 b
KS 6	52.990±3.59 a	8.128±0.20 c	16.698±1.14 a	0.921±0.04 bc
KS 8	33.462±0.47 b	6.914±0.51 c	11.976±0.19 bc	0.765±0.005 c
KS 15	44.464±0.41 ab	14.228±1.09 a	16.997±0.76 a	0.926±0.04 bc
KS 17	34.056±1.21 b	9.324±1.03 bc	11.959±0.96 bc	0.895±0.001 bc
KS 20	39.190±4.25 b	15.319±0.38 a	14.555±0.13 ab	0.457±0.01 d

Mean values (Mean ± SE) in the same column but assigned different letters (a–d) are significantly different according to Tukey's range test at P≤0.05.

Table 9. Total polyphenol content and antioxidant capacity.

Biotypes	Total without anthocyanins (µg catechin/g skin)	Total with anthocyanins (µg catechin/g skin)	Total with anthocyanins (µg gallic/g skin)	Flavonoids (µg/g skin)	Antioxidantcapacity FRAP (mg Trolox/g FW)	Antioxidantcapacity DPPH (mg Trolox/g FW)
KS 1	5231.63±56.37 a	29335.15±13 abc	5114.3±240.77 ab	5280.88±252.17 b	24.09±0.01 bc	14.57±0.23 ab
KS 2	3846.831±113.84 b	30025.3±159 ab	5234.64±278.07 ab	5373.53±213.96 b	26.26±1.74 ab	13.26±0.53 bc
KS 6	3262.73±7.40 bc	26306.9±403.4 ab	4586.37±70.33 ab	5519.11±236.88 b	23.55±0.53 bc	11.66±0.13 cd
KS 8	3266.36±21.87 bc	32891.9±940.9 a	5734.39±164.03 a	6247.05±152.83 a	21.22±0.01 cd	11.37±0.23 d
KS 15	3762.62±106.15 b	33792.2±4234.1 a	5891.35±738.17 a	6670.59±259.81 a	28.8±0.50 a	14.4±0.19 ab
KS 17	3762.62±355.16 d	27921.4±554.2 ab	4867.83±96.62 ab	5585.29±137.55 b	26.06±1.18 ab	15.54±0.33 a
KS 20	2974.98±41.75 cd	23258±637.7 b	4054.81±111.19 b	3970.58±137.55 c	18.18±0.12 d	11.2±0.59 d

Mean values (Mean ± SE) in the same column but assigned different letters (a–d) are significantly different according to Tukey's range test at P≤0.05.

Table 10. Principal components (PCs) for 45 variables/measurements of the 7 biotypes evaluated.

Variables/Measurements	Eigenvectors/Principal Components						
	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Gallic acid	0.074	0.027	-0.281	0.036	0.200	-0.209	0.074
Protocatechuic acid	-0.004	-0.031	-0.259	0.115	0.176	-0.259	-0.051
Procyanidin B1	0.050	0.075	-0.245	-0.014	0.263	-0.041	0.211
Caftaric acid	0.218	-0.205	0.038	0.083	0.050	0.044	-0.064
Catechin	0.160	-0.166	0.160	0.119	-0.095	-0.215	-0.005
Procyanidin B2	0.036	-0.016	-0.292	0.154	0.061	0.112	-0.095
Vanillic acid	-0.030	-0.058	-0.224	0.170	0.015	0.366	-0.064
Caffeic acid	0.228	-0.198	0.046	0.061	0.058	-0.055	-0.033
Coutaric acid	0.214	-0.197	0.070	0.097	0.060	-0.026	-0.051
Vanilin	0.245	-0.046	-0.042	0.206	-0.062	-0.054	0.055
Syringic acid	-0.010	0.197	-0.150	0.235	-0.069	-0.045	-0.120
Fertaric acid	0.147	-0.194	0.089	0.204	-0.142	0.020	-0.095
Epicatechin	-0.020	0.113	-0.133	0.222	-0.194	-0.265	0.164
P-coumaric acid	-0.111	0.225	-0.073	0.126	0.003	-0.016	-0.143
Piceid	-0.001	-0.096	-0.083	0.101	0.432	0.103	-0.201
Ferulic acid	0.156	0.168	0.120	0.049	0.231	0.202	-0.013
Sinapic acid	-0.228	0.121	0.107	0.069	0.154	0.125	0.024
m-coumaric acid	0.046	0.187	0.057	0.301	-0.045	0.098	-0.007
Rutin	0.139	-0.231	-0.002	0.162	-0.187	0.014	-0.071
Trans-resveratrol	0.044	-0.199	-0.081	0.191	0.289	-0.021	-0.027
ε-Viniferin	-0.058	0.032	0.262	-0.104	0.258	0.142	-0.040
Quercetin	-0.003	-0.221	0.201	-0.009	0.163	0.140	-0.037
Luteolin	-0.224	0.019	0.141	-0.010	0.190	0.221	-0.051
Total phenols without anthocyanins	0.175	-0.245	0.072	0.072	-0.017	0.073	-0.077
Total phenols with anthocyanins	0.180	0.028	-0.074	-0.036	-0.112	0.342	0.339
Total flavanoids	0.195	0.127	-0.151	-0.046	-0.011	0.213	-0.182
Delphinidin	0.203	0.187	0.085	-0.118	0.073	-0.118	-0.072
Cyanidin	0.100	0.283	-0.057	-0.090	0.003	0.031	-0.083
Petunidin	0.199	0.201	0.102	-0.089	0.061	-0.097	-0.072
Peonidin	0.195	0.218	0.098	-0.047	0.033	-0.007	-0.102
Malvidin	0.190	0.194	0.162	-0.011	0.044	-0.105	-0.048
Total anthocyanins	0.203	0.198	0.108	0.016	0.019	-0.024	0.162
Total flavanols	0.258	0.089	-0.091	0.001	0.077	0.032	0.055
Average length of grape	-0.063	-0.015	0.064	0.214	0.080	0.062	0.438
Average width of grape	-0.050	-0.098	0.273	0.036	0.158	-0.025	0.156
Average weight of grape	-0.090	0.003	0.098	0.259	0.092	-0.112	0.393
Average weight of 50 berries	-0.150	0.024	0.143	0.268	-0.075	0.036	-0.150
pH	0.017	-0.101	-0.103	-0.184	-0.307	0.258	-0.081
Total titratable acidity	0.024	0.059	0.288	-0.017	-0.183	-0.111	0.052
Total soluble solids	0.002	-0.132	-0.263	-0.197	0.005	-0.066	0.049
Average length of berry	-0.191	0.095	-0.004	0.241	-0.087	0.081	-0.060
Average width of berry	-0.158	0.104	-0.017	0.260	-0.138	0.091	-0.031
DPPH	0.122	0.132	0.099	0.269	-0.061	0.079	-0.241
FRAP	0.226	0.155	0.031	0.116	0.099	0.072	0.075
Eigenvalue	10.997	9.023	8.110	6.047	3.548	2.636	1.510
Individual variation explained	24.4	20.1	18.0	13.4	7.9	5.9	3.4
Cumulative variation explained	24.4	40.1	58.1	71.6	79.4	85.3	88.7

concentrations in malvidin, but no statistically significant difference was observed between their concentrations (Table 5). These results confirm the findings of previous studies (Boss et al., 2009; Chiou et al., 2014) that malvidin seems to be the dominant anthocyanin. Furthermore, another study has found malvidin to predominate in grapes of grapevine varieties cultivated in and native to Greece, followed by peonidin, petunidin, delphinidin, and cyanidin (Biniari et al., 2018).

Total flavanols and total flavonoids: Biotype KS15 exhibited the highest concentration in total flavanols and total flavonoids, with no statistically significant differences either in total flavanols when compared to biotypes KS2 and KS6; or total flavonoids when compared to biotype KS8. It was biotype KS20 which registered the lowest concentration in total flavanols and total flavonoids and showed statistically significant differences from all other biotypes studied (Tables 4, 9).

Non-flavonoids

Hydroxybenzoic acids: Gallic, protocatechuic, vanillic and syringic. All acids cited were identified and quantified in the Korinthiaki Staphis biotypes studied. Biotype KS6 exhibited the highest concentration in gallic and protocatechuic acid, with a statistically significant difference from the remaining biotypes (Table 6). KS8 scored the highest concentration in vanillic acid without, however, any statistically significant difference when compared to biotypes KS1, KS15, and KS7. Biotype KS17 presented the highest concentration in syringic acid but with no statistically significant difference when compared to biotype KS6. All results agree with the findings of previous studies on the same acids (Williamson and Carughi, 2010; Meng et al., 2011).

Hydroxycinnamic acids: Sinapic, caffeic, p-coumaric, m-coumaric, ferulic, caftaric, coutaric, and fertaric. All hydroxycinnamic acids cited were identified and quantified in all Korinthiaki Staphis biotypes studied. Biotype KS1 exhibited the highest concentrations in caffeic, caftaric, coutaric, and fertaric acid, registering a statistically significant difference in comparison to the same hydroxycinnamic acids in the remaining biotypes (Table 7). At this point, it is worth noting the importance of the presence of caftaric acid at relatively high concentrations in all biotypes studied. Apart from being a polyphenolic compound with an antioxidant capacity, it is generally not affected by the drying process (Karadeniz et al., 2000). Consequently, the raisins those biotypes yield are expected to enjoy remarkable quality.

Stilbenes - Phenolic aldehyde: Resveratrol has drawn considerable scientific interest ever since its anticarcinogenic properties were detected (Jang et al., 1997; Schneider et al., 2000). Resveratrol was identified and quantified in all biotypes studied at satisfactory concentrations, ranging from 31.7 to 56.3 μg/g skin (Table 8). Moreover, the values recorded by the present study are higher than those quantified in currants in the study of Chiou et al. (2007); or the ones in the study of Karadeniz et al. (2000) which pointed at the absence of resveratrol in raisins. Piceid and ε-viniferin were also identified and quantified in all biotypes studied. The concentration of the three stilbenes (trans-resveratrol, ε-viniferin, piceid) proved to be lower than that of wine grape varieties investigated by previous studies (Anastasiadi et al., 2010; Biniari et al., 2018). Biotype KS1 scored the highest concentration in trans-resveratrol and a significantly high concentration in piceid. Equally high and with a statistically significant difference from the other biotypes (Table 8) was the concentration of the same biotype in vanillin, the phenolic aldehyde which influences aroma (Moreno-Arribas and Polo, 2009).

Antioxidant capacity

The DPPH free radical scavenging effect of extracts was calculated and differences in DPPH between the different biotypes were observed: biotype KS17 exhibited the highest value (15.54 mg Trolox/g FW), followed by KS1 and KS15. Biotypes KS8 and KS20 exhibited the lowest scavenging capacity (11.37 and 11.2 mg Trolox/g FW, respectively). With respect to FRAP, biotype KS15 (28.8 mg Trolox/g FW) exhibited the highest reducing power without a significant difference when compared to biotypes KS2 and KS17 (Table 9). Further, a significantly negative correlation was observed between the values obtained for DPPH and FRAP assays, stemming from the fact that each test measures different routes for the antioxidant action of polyphenols (Fabani et al., 2017): FRAP assays measure the extract's reducing power, while DPPH ones evaluate the extract's ability to quench free radicals.

Estimation of total polyphenol content

The total polyphenol content of the seven biotypes studied (Table 9), was estimated according to the Illand assay, and expressed as μg gallic acid/g; and as mg catechin/g of berry skin. Biotype KS1 exhibited the highest concentration in total polyphenol content without anthocyanins (expressed as mg catechin/g skin); and a statistically significant difference from the remaining biotypes. After anthocyanins were expressed both as mg catechin/g skin; and as mg gallic acid/g skin, the total polyphenol content registered no statistically significant differences between the biotypes studied, with the exception of (a) biotype KS20, which exhibited the lowest concentration; and (b) biotype KS15, which presented the highest concentration. The above results suggest, as did a previous study (Di Lorenzo et al., 2016), that biotypes with a high concentration in total polyphenol content will most likely yield raisins of higher quality.

Principal Component Analysis (PCA)

PCA transforms the original data set, all measurements included, into a smaller set of uncorrelated new variables (Principal Components, where eigenvalues were>1). When performed on the biotypes studied, it produced seven (7) components in a declining order of importance which accounted for and explained 93.048% of the total variability among the different biotypes. All measurements grouped under the same principal component show a strong correlation between them. So as to estimate the initial measurements' contribution to variability, each component strongly correlates with a set of the initial measurements. Measurements which strongly correlated with the first seven (7) components are presented in Table 10 and Fig 1. For example, the measurements gauging total flavanols, vanillin, caffeic acid, sinapic acid, FRAP, luteolin, caftaric acid, coutaric acid, total anthocyanins, and delphinidin in the biotypes studied contributed more satisfactorily to variability than measurements taking into account the average length and/or weight of grape.

Correlation between antioxidant capacity and polyphenolic profile

The antioxidant capacity has a direct correlation with the polyphenolic profile (Balík et al., 2008; Garaguso and Nardini, 2016). As also confirmed by the PCA (Fig 1), the pairwise correlation analysis and linear correlation of all measurements showed that, anthocyanins (total and individual), total flavonoids, and total flavanols strongly and positively correlate (P<.0001) with antioxidant capacity (FRAP, DPPH). On the other hand, total soluble solids compounds correlated negatively (P<-.0005) with antioxidant capacity (DPPH). Moreover, as expected, it was

confirmed that the weight of berries of the biotypes studied correlates negatively with total flavanols, total soluble solids, and pH, since these measurements are affected by the ratio skin:flesh.

Materials and methods

Plant materials

Table 1 shows seven (7) biotypes of the grapevine variety Korinthiaki Staphis (*Vitis vinifera* L.). Those were selected for study via an assay of their polyphenolic profile on the basis of (a) their being the most representative ones among numerous other biotypes of the variety at main cultivation centers; (b) their morphology; and (c) their productivity. All biotypes were identified by means of the ampelographic description and of molecular methods. The experimental vineyard, where the biotypes are preserved and where form the samples were collected (Stavrakaki and Biniari, 2016), is located in Nemea (alt: 195-200 m), northeastern Peloponnese, Greece. The seven-year-old vines were all grafted on rootstock 1103 Paulsen; were bilateral cordon-trained (bilateral Royat) at 2.2m x 1.2m intervals; and were spur-pruned to 2-node spurs. The usual viticultural techniques were applied, i.e., fertilization using 11-15-15 NPK at a dose 250 g/vine; canopy management techniques (shoot thinning, topping; girdling); and irrigation. All biotypes studied were grown in the same area and under the same conditions. The experiment took place during the 2016 cultivation season. Since an additional goal of the study was to evaluate the biotypes' early/late ripening, the harvest of the grapes of all biotypes studied took place on the same day, namely, August 24, 2016. Grapes were randomly selected from different vines of each biotype and three (3) sampling processes took place. The grapes were collected from the main shoots of different positions. Each sampling constituted one replication. A total of three (3) replications per treatment (biotype) took place.

Reagents and chemicals

The various polyphenolic compounds analyzed were identified according to their order of elution and the retention times of the pure compounds. Anthocyanins (delphinidin-3-O-glucoside, cyanidin-3-O-glucoside, petunidin-3-O- glucoside, peonidin-3-O-glucoside, malvidin-3-O-glucoside) were all purchased from Extrasynthese, Gemay, France. However, non-colored polyphenolics were purchased from a number of different sources. More specifically, gallic acid, protocatechuic acid, catechin, vanillic acid, caffeic acid, syringic acid, vanillin, epicatechin, ferulic acid, sinapic acid, m-coumaric acid, p-coumaric, and rutin were purchased from Sigma, St. Louis, MO, USA; luteolin, procyanidin B1, procyanidin B2, ε-viniferin, quercetin, trans-resveratrol, and piceid were purchased from Extrasynthese, Gemay, France; and coutaric acid, caftaric acid, and fertaric acid were purchased from PhytoLab GmbH & Co. KG, Vestenbergsgreuth, Germany.

Bunch and berry mechanical properties (weight, length, and width)

Nine (9) grapes were collected from different vines of each biotype. The weight of each one of the grapes was measured using a precision scale. The grapes' length and width were determined using calipers of an 0.01 mm accuracy. Three (3) random groups of fifty (50) berries were collected from the grapes of each biotype. Each group's weight was measured using a precision scale. The ensuing weight was then divided by the number of berries. Next, the mean weight of each berry was calculated per group. The length and width of each berry in all three (3) groups were measured using a Vernier caliper. Last, the mean value of each group's berry length and width was calculated.

Determination of soluble solids, pH and total titratable acidity

Soluble solids in must were determined using an ATAGO N1-a refractometer with a 0-32 Brix measurement range at 0.28 Brix increments, and no temperature compensation. Total titratable acidity was measured by titration with a 0.1 N NaOH solution. Total titratable acidity was expressed as tartaric acid, the organic acid most abundant in *Vitis vinifera* grapes.

Sample preparation for spectrophotometric and HPLC analysis

For each replication and in order to separate skin from berry, approximately one hundred (100) berries were peeled by hand. Next, the peeled skins were dried in a freeze drier through lyophilization and then pulverized in a mill. Last, the samples (skin powder) were preserved in deep freeze, at a temperature of -80°C. Preparation of the berry skin extracts: 0.4 g dried skins (skin powder) were mixed with 4 mL extraction medium water/methanol/acetone/HCl (19/40/40/1), homogenized for one (1) min at 8000 rpm in an Ultra Turrax homogenizer. The extracts were shaken in a controlled-temperature vacuum at 150 rpm for thirty (30) min at a room temperature of 25°C. Samples were centrifuged at 4500 rpm for ten (10) minutes. Supernatants were collected, and the extraction procedure was repeated twice more. All fractions were combined, and the supernatants were stored at a temperature of -80°C until the time of the analysis.

Determination of total phenols and total anthocyanins

Total phenols and total anthocyanins were measured using the Somers and Evans (1977) methods, albeit with some modifications (Biniari et al., 2018).

Determination of total flavonoid content

Total flavonoid content was determined using a colorimetric method described by Dewanto et al., (2002). Absorbance was immediately measured against the blank at 510 nm.

Concentration of total flavanols was estimated from a calibration curve, constructed by plotting known solutions of catechin (12.5-200 µg/mL). The results were expressed as mean (milligrams of catechin equivalent per gram of skin).

Determination of total flavanols

The total flavanol content was estimated using the p-dimethylaminocinnamaldehyde (DMACA) method (Vivas et al., 1994; Li et al., 1996; McMorrough et al., 1996). The concentration of total flavanols was estimated from a calibration curve, constructed by plotting known solutions of catechin (12.5-200 µg/mL). The results were expressed as mean (milligrams of catechin equivalent per gram of skin).

Analysis by HPLC

Anthocyanins and polyphenols were analyzed using the high-performance liquid chromatography (HPLC) method. The identification was based on comparing retention times and on-line spectral data in comparison to original standards. The quantification was performed using the calibration curves of each standard compound. The concentration was estimated from a calibration curve, constructed by plotting known solutions (1.25–20 µg/mL). The results were expressed as mean (micrograms of catechin equivalent per gram of skin). The analyses were performed using an HPLC Shimadzu Nexera comprising a gradient pump Shimadzu Nexera X2, a ProStar model 410 AutoSampler, and a ProStar model 330 Photodiode Array Detector on a reversed-phase Waters C18 x select (250 mm x 4.6 mm, 5 mm) column at a temperature of 25°C.

HPLC analysis of anthocyanins

For the measurement of anthocyanins with HPLC, 1 mL of the supernatant was evaporated with a sample concentrator at room temperature under a stream of nitrogen gas. The pellet was dissolved in 20 mL of 50% methanol in water. Anthocyanins (delphinidin-3-O-glucoside, cyanidin-3-O-glucoside, petunidin-3-O-glucoside, peonidin-3-O-glucoside, malvidin-3-O-glucoside) were determined by the HPLC-DAD system (Shimadzu Nexera). For the separation of monomeric anthocyanins, a 250x4.6 mm i.d., 5 µm, Waters x select C18 column was employed operating at 25°C. The eluent was composed of (a) H_2O/HCOOH (90:10); and (b) CH_3OH (100). The flow rate stood at 1 mL/min. For the elution, the following linear gradient program was used: 5% B for 0 min; 5%-50% B in 25 min; 50%-95% B in 30 min; followed by a return to the initial conditions in ten (10) minutes and re-equilibration of the column. The chromatogram was monitored at 520 nm.

HPLC analysis of individual polyphenols

In order to measure individual polyphenols with HPLC, liquid extraction was carried out. An extract of 0.5 mL was mixed up with 4 mL ethyl acetate with vortex, and the supernatant was separated. The supernatant was then washed twice with distilled water. Next, the supernatants were evaporated with a sample concentrator, and the pellets were dissolved in 1 mL of 50% methanol in water. Last, prior to the HPLC analysis, 1.5 mL was filtered through a 0.22 µm membrane. The following were determined using the HPLC-DAD system (Shimadzu Nexera): monomeric and dimeric polyphenols (+)-catechin, (-)-epicatechin, proanthocyanidins B1 and B2, gallic acid, protocatechuic acid, caftaric acid, vanillic acid, caffeic acid, coutaric acid, vanillin, syringic acid, fertaric acid, p-coumaric, m-coumaric, piceid, ferulic acid, sinapic acid, rutin, trans-resveratrol, ε-viniferin, quercetin, and luteolin.

For the separation of monomeric and dimeric polyphenols, the study employed a 250x4.6 mm ID, 5 µm, Waters x select C18 column operating at 25°C. The eluent was composed of (a) H_2O/$HClO_4$ (99:1) and (b) CH_3OH (100). The flow rate was 0.5 mL/min. The following linear gradient program was used for the elution: 0% B for 2 min, from 0%-5% B in 16 min; from 5%-10% B in 25 min; from 10%-15% B in 50 min; from 15%-25% B in 90 min; from 25%-45% B in 120 min; from 45%-75% B in 145 min; from 75%-90% B in 150 min; and from 90%-95% B in 155 min, followed by a return to the initial conditions in ten (10) minutes and re-equilibration of the column. The chromatogram was monitored at 280, 320, and 360 nm.

Determination of antioxidant activity by the DPPH radical scavenging method

Antioxidant activity (2,2-diphenyl-1-picrylhydrazyl, DPPH) was evaluated by the free radical scavenging activity of DPPH using a modified colorimetric method proposed by Brand-Williams et al. (1995). The scavenging activity of the DPPH radical was determined using as a standard 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) (Sigma Chemical Co.). The standard curve was linear between 2.0 and 1598.1 µM Trolox. The results were expressed as mg Trolox per gram of skin.

Ferric Reducing Antioxidant Power (FRAP)

For the Ferric Reducing Antioxidant Power (FRAP), the protocol described by Benzie and Strain (1996) was used. The results were expressed as mg Trolox per gram of skin.

Data analysis

All results were expressed as mean ± SE (Standard Error) of the three (3) replications out of three (3) samples/grapes. Note that the three grapes counted as one replication. All determinations were analyzed in triplicate. Data were processed by Analysis of Variance (ANOVA). The statistical significance was processed using Tukey's range test at $P \geq 0.05$. Principal Component Analysis (PCA) was used in evaluating the measurements and their contribution to the variability of the biotypes studied. All statistical analysis and correlations were obtained using the JMP v.10 statistical software (SAS Institute Inc., Cary, NC, USA).

Conclusion

The biotypes with the most abundant polyphenol content also showed the highest antioxidant activities, thus confirming previous studies (Di Lorenzo et al., 2016). In the present study, it was observed that, in all Korinthiaki Staphis biotypes studied, the antioxidant capacity is dependent on the polyphenolic compounds and positively correlates with

mainly total polyphenolics, total anthocyanins, and total flavanols. As far as the polyphenolic compounds and antioxidant capacity are concerned, the biotypes (clones) studied appear to have the potential to improve the quality of such products as raisins and wine which derive from them. The present study's results also showed that both polyphenolic content and its antioxidant capacity are biotype dependent. Consequently, it would be of major importance to viticulturists to exploit the appropriate biotypes of Korinthiaki Staphis in order to obtain higher quality products.

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